REMARKS

Applicants wish to thank the Examiner for the attention accorded to the instant application, and respectfully requests reconsideration of the application as amended. Claims 1-18 are pending in the present application. Claims 1 and 18 have been amended to recite that the analyzing step of measuring the differences in molecular weight between the series of reaction products and the original peptide is by means of MALDI-TOF-MS. Support for this amendment can be found throughout the instant application generally, see for example page 44 line 23 to page 45 line 10. Claims 1, 5, 6, 7 and 18 have been amended to recite that the molecular weight measurement is for the cationic species of $(M+H)^+$ as well as for the anionic species of $(M-H)^-$. Support for these amendments can be found throughout the application generally, table 4 specifically. Claim 4 has been amended to correct an informality. In addition the claims have been amended to correct grammatical errors.

Claims 1, 2, and 4-6 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Tsugita, Akira et. al., "Additional Possible tools for identification of proteins on one or two dimensional electrophoresis, 1998, Electrophoresis, Vol. 19, pages 928-938 (hereinafter "Tsugita") in view of Covey et. al. U.S Patent No. 5,952,653 (hereinafter "Covey") and Xu, Naxing et. al., "Structural characterization of peptidoglycan muropeptides by matrix-assisted laser desorption ionization mass spectrometry and postsource decay analysis," 1997, Analytical Biochemistry, Vol. 248, page 7-14 (hereinafter "Xu").

Claim 3 stands rejected under 35 U.S.C. 103(a) as being unpatentable over Tsugita in view of Covey and Xu, as applied to Claims 1-2 and 4-6 above, and further in view of Harris, William A., et. al., "Use of matrix clusters and trypsin autolysis fragments as mass calibrants in

matrix assisted laser desorption/ionization time-of-flight mass spectrometry," 2002, Rapid Communications in Mass Spectrometry, vol. 16, pages 1714-1722 (hereinafter "Harris").

Claims 7-17 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Tsugita in view of Covey and Xu as applied to 1, 2 and 4-6 above, and further in view of Vogt, S. et. al., "Effective esterification of carboxymethyl cellulose in a new non-aqueous swelling system," 1996, Polymer Bulletin, Vol. 36, page 549-555 (hereinafter "Vogt").

In view of the following remarks, Applicants request further examination and reconsideration of the present patent application.

Rejections under 35 U.S.C. §103

Claims 1, 2, and 4-6 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Tsugita in view of Covey and Xu. This rejection should be withdrawn based on the comments and remarks herein.

The combination of Tsugita, Covey and Xu do not render the claimed invention obvious for at least the following reasons. None of the references teach or suggest measuring the mass/charge of the cationic species of $(M+H)^+$ as well as the mass/charge measurement for the anionic species of $(M-H)^-$, for determining an amino acid sequence of an unknown polypeptide, as recited in Claims 1, 18 and all claims depending therefrom of the present application.

Tsugita teaches identification of proteins with the use of mass spectrometry. See Abstract of Tsugita. Tsugita further teaches a method of measuring the protonated molecules $[M+H]^+$ of fragments after tryptic digestion. See page 932 left column first paragraph of Tsugita. But, Tsugita does not teach, disclose or suggest the use of anionic species for determining the sequence. More specifically, a review of Tsugita clearly reveals that it is completely silent to the use of an anionic species. Thus, Tsugita does not teach, disclose or

suggest the use of an anionic species for determining the amino acid sequence of a polypeptide, as claimed. Therefore, Tsugita does not teach measuring the mass/charge of the cationic species of $(M+H)^+$ as well as the mass/charge measurement for the anionic species of $(M-H)^-$, as recited in Claims 1, 18 and all claims depending therefrom of the present application.

Covey teaches a double charge rule that the tryptic fragment having Arg or Lys at the C-terminus thereof will be doubly positively charged in such form of $(M+2H)^{2+}$ by using Ion spray process for Ion Evaporation Mass Spectrometry, but that there are three exceptions to the double charge rule as follows:

First exception: a tryptic fragment having other amino acids than Arg or Lys at the C-terminus thereof will only be singly charged in such form of $(M+H)^+$ by using Ion spray process for Ion Evaporation Mass Spectrometry.

Second exception: a tryptic fragment having an amino terminus which is carboxylated or blocked (e.g. N-acylation at the N-terminus) will only be singly charged in such form of $(M+H)^+$ by using Ion spray process for Ion Evaporation Mass Spectrometry.

Third exception: a tryptic fragment having Arg or Lys at the C-terminus thereof and containing an internal His will be triply charged in such form of $(M+3H)^{3+}$ in small percentage, but will be doubly charged in such form of $(M+2H)^{2+}$ in most percentage. See column 5 line 53 to column 6 line 8 of Covey.

Covey fails to provide any teaching or suggestion as to whether or not such a double charge rule will be also observed for MALDI-TOF-MS or FAB-MS. Covey also fails to teach or suggest that a singly positive charged ion of $(M+H)^+$ from the tryptic fragment having Arg or Lys at the C-terminus thereof may show stronger intensity in the spectrum of the cationic species of $(M+H)^+$ measured by MALDI-TOF-MS.

Again, Covey, like Tsugita does not utilize any anionic species. A review of Covey clearly shows that he does not utilize any anionic species in his analysis.

Further, Covey fails to provide any suggestion as to intensity of a singly positive charged ion of $(M+H)^+$ and a singly negative charged ion of $(M-H)^-$ from the tryptic fragment having Arg or Lys at the C-terminus thereof to be measured by MALDI-TOF-MS, as recited in Claim 1, 18 and all claims depending therefrom of the present application.

Xu teaches a procedure of structural characterization of muropeptides derived from peptidoglycan by means of a MALDI-MS based method.

Xu teaches a method where first, the monomeric and dimeric muropeptides are previously identified by FAB-MS and FAB-MS/MS. In addition, the amino acid compositions of each of the muropeptides were determined in advance by mass-spectrometry. Thus, the structures of pentaglycine-substituted monomer and the muropeptide oligomers can be reasonably predicted on the basis of the reported structures of the monomeric and dimeric muropeptides determined by the previous mass-spectrometry step. To do the initial mass-spectrometry step using a FAB-MS or FAB-MS/MS, Xu teaches that the muropeptide samples were digested with lysostaphin in 12.5 mM sodium phosphate buffer (pH 5.5) for 16 hours and analyzed directly without further purification. Thus, the analyte solution used for MALDI analysis contains a mixture of the fragments having N-terminal amino group of a Gly residue included in a polyglycine chain substituted on the Lys residue and the C-terminal carboxyl group of Ala (or Gly) at the C-terminus of the main peptide chain in the 12.5 mM sodium phosphate buffer. The samples for mass spectrometric analysis were typically prepared by depositing about 1 μL of CMBT solution and about 1 μL of the analyte solution in a sample plate well and mixing. The solvents were removed by air drying. In the dried-up samples, most of the

fragments may be present in a salt form in which the C-terminal carboxyl group of Ala (or Gly) at the C-terminus of the main peptide chain is converted into $-COO^-Na^+$, whereas minor fragments may be present in a form in which the C-terminal carboxyl group of Ala (or Gly) at the C-terminus of the main peptide chain is converted into -COOH.

In Xu, the singly ionized cationic species of $[M+Na]^+$ is predicted to be a cation in which the N-terminal amino group of the Gly residue included in the polyglycine chain is ionized in the form of $-NH_3^+$, and the C-terminal carboxyl group of Ala (or Gly) at the C-terminus of the main peptide chain is converted into $-COO-Na^+$.

In this method, a protonated molecule of $(M+H)^+$ is predicted to be a cation in which the N-terminal amino group of Gly residue included in polyglycine chain is ionized in the form of $-NH_3^+$, and the C-terminal carboxyl group of Ala (or Gly) at the C-terminus of the main peptide chain is present in the form of -COOH. The singly ionized anionic species of $(M-H)^-$ is predicted to be a cation in which the N-terminal amino group of the Gly residue included in the polyglycine chain is present in the form of $-NH_2$, and the C-terminal carboxyl group of Ala (or Gly) at the C-terminus of the main peptide chain is ionized in the form of $-COO^-$.

According to the method of Xu, in the Negative-ion MALDI mass spectrum of the lysostaphin digest of multipeptides, only the single peak generated from each of the fragments is observed at the position of m/z=(M-1). On the other hand, in the positive-ion MALDI mass spectrum of the lysostaphin digest of multipeptides, the series of peaks of the singly ionized cationic species of $[M+Na]^+$ and the series of peaks of the singly ionized cationic species of $(M+H)^+$ will be both observed at the position of m/z=(M+23) and at the position of m/z=(M+l) in parallel.

Therefore, the Negative-ion MALDI mass spectrum of the lysostaphin digest of multipeptides is suitably used to identify the mass of each of the fragments contained in the analyte solution, by referring to the well predicted structures of muropeptides, as illustrated In FIG. 6 and TABLE 1 and in FIG. 7 and TABLE 2 of Xu.

In addition, collected muropetides that are separated by HPLC were desalted with acetonitrile, and thus, the desalted muropeptides samples are present in such a form in which the N-terminal amino group of Gly residue included in the polyglycine chain is present in the form of $-NH_2$, and the C-terminal carboxyl group of Ala (or Gly) at the C-terminus of the main peptide chain is present in the form of –COOH.

Therefore, in the positive-ion MALDI mass spectrum of the desalted muropeptides of Xu, only a single peak of the singly ionized cationic species of $(M+H)^+$ will be observed at the position of m/z=(M+1), but any peak of a singly ionized cationic species of $[M+Na]^+$ may not be observed. In the negative-ion MALDI mass spectrum of the desalted muropeptides in Xu, only a single peak of the singly ionized anionic species of $(M-H)^-$ will be observed at the position of m/z=(M-1). In particular, in MALDI-PSD analysis of an un-substituted monomer, Xu clearly states "In positive-ion mode analysis, the $[M+Na]^+$ ion at m/z 991 was selected as the precursor for an un-substituted muropeptide monomer because of its high abundance relative to the protonated molecules $(M+H)^+$." See page 10 right column of Xu.

Accordingly, the molecular weight of the desalted muropeptide dimer I was determined as 2417 by both positive and negative-mode MALDI-MS, with ease. However, Xu fails to utilize $(M+H)^+$ or $(M-H)^-$ for each fragment. Xu is limited to the use of the negative ions for determining the molecular weight of the muropeptide. Xu is limited to the use of $(M-H)^-$ value for the molecular weight and nothing more. Thus, Xu fails to suggest that the spectrum observed

in the positive mode would be suitably used in combination with the spectrum observed in the negative mode to identify the mass of each of the fragments contained in the analyte solution.

Accordingly, Xu fails to suggest any use of the protonated molecules $(M+H)^+$ for the positive-ion mode analysis. Moreover, Xu does not utilize $(M-H)^-$ values for the anionic species for each fragment. Further, Xu only teaches the use of both the positive and negative mode MALDI-MS to determine molecular weight, not mass per charge or intensity. See page 12 left column first paragraph of Xu.

Accordingly, Xu fails to provide any teaching or suggest measuring the mass per charge of the cationic species of $(M+H)^+$ as well as the mass per charge for the anionic species of $(M-H)^-$, as recited in Claims 1, 18 and all claims depending therefrom of the present application. Thus, the combination of the cited references fails to teach the use of the measurement of the mass/charge for each of the cationic species of $(M+H)^+$ as well as the use of the mass/charge measurement for each of the anionic species of $(M-H)^-$ being generated from ionization treatment by means of MALDI-TOF-MS. The measurement of the mass/charge of both of the cationic species of $(M+H)^+$ and the anionic species of $(M-H)^-$, as recited in Claims 1, 18 and all claims depending therefrom of the present application, is advantageous and provides a more accurate determination of peptide fragments, as shown by comparing the methodology of the present invention with the methodology of the combination of the cited references.

The claimed invention recites the measurement of the mass/charge of the cationic species of $(M+H)^+$ as well as the mass/charge measurement for the anionic species of $(M-H)^-$. Prior to measurement by MALDI-TOF-MS, a peptide fragment having a molecular weight M is subject to ionization treatment. Singly ionized cationic species of $(M+H)^+$, the doubly ionized cationic

species of $(M+2H)^{2+}$, and the singly ionized anionic species of $(M-H)^-$ are very often generated. The doubly ionized anionic species of $(M-2H)^{2-}$ is rarely generated.

When a peptide fragment having a molecular weight of M is subjected to the ionization treatment by means of MALDI-TOF-MS, the singly ionized cationic species of $(M+H)^+$, the doubly ionized cationic species of $(M+2H)^{2+}$ and the singly ionized anionic species of $(M-H)^-$ are very often generated from the peptide fragment of M, but the doubly ionized anionic species of $(M-2H)^{2-}$ are rarely generated from the peptide fragment of M. In the spectrum of the cationic species measured by means of MALDI-TOF-MS, the singly ionized cationic species of $(M+H)^+$ is measured at m/z=M+1 with considerable intensity, whereas the doubly ionized cationic species of $(M+2H)^{2+}$ is measured at m/z=(M+2)/2 with considerable intensity. In the spectrum of the anionic species measured by means of MALDI-TOF-MS, the singly ionized anionic species of $(M-H)^-$ is measured at m/z=M-1 with considerable intensity whereas the doubly ionized anionic species of $(M-2H)^{2-}$ is measured at m/z=(M-2)/2 with very weak intensity.

Although methods of the present invention and that of the combination of the prior art may produce the same species, as described below, only the method of the present invention will correctly assign all of the fragments.

In the method, as suggested by the combination of Tsugita, Covey and Xu, if the peak of the cationic species is measured in the spectrum of the cationic species with considerable intensity and the peak of the anionic species is measured in the spectrum of the anionic species with considerable intensity, the peak of the cationic species is reasonably assigned as the peak of singly ionized cationic species generated of $(M+H)^+$ from the peptide fragment of M, and the peak of the anionic species is also assigned as the peak of singly ionized anionic species of $(M-H)^-$ generated from the peptide fragment of M.

In a method, as suggested by the combination of Tsugita, Covey and Xu, where only the spectrum of one species is measured, there are many shortcomings. If the peak of the cationic species is measured in the spectrum of the cationic species with considerable intensity, and the peak of the anionic species is measured in the spectrum of the anionic species with very weak intensity, the peak of the cationic species is reasonably assigned as the peak of doubly ionized cationic species of $(2M+2H)^{2+}$ generated from the peptide fragment of 2M, and the peak of the anionic species is also reasonably assigned as the peak of doubly ionized anionic species of $(2M-2H)^{2-}$ generated from the peptide fragment of 2M.

The groups of the peaks of singly ionized cationic species having considerable intensity are reasonably selected from the mixture of the peaks of singly ionized cationic species and doubly ionized cationic species having considerable intensity observed in the spectrum of the cationic species. Further, groups of the peaks of singly ionized anionic species having considerable intensity are reasonably selected from the mixture of the peaks of singly ionized anionic species and doubly ionized anionic species observed in the spectrum of the cationic species.

In a method where only the spectrum of the cationic species are measured by means of MALDI-TOF-MS, it is very difficult to reasonably distinguish distinct groups of the peaks of the singly ionized cationic species having considerable intensity form the group of peaks of doubly ionized cationic species having considerable intensity. In a method where only the spectrum of the anionic species are measured by means of MALDI-TOF-MS, it is hard to reasonably distinguish distinct groups of the peaks of singly ionized anionic species from groups of the peaks of doubly ionized anionic species. In the method of the combination of the prior art, it is still difficult to

reasonably distinguish distinct groups of the singly ionized species from the species of doubly ionized species.

In another example when a peptide fragment with molecular weight of M having N-terminal amino group ($-NH_2$) and Arg as its C-terminal amino residue: H_2N-A_1-A_2-...-A_{i-1}-Arg, or the N-terminal acetylated peptide fragment with molecular weight of M having Arg as its C-terminal amino residue: CH_3CO-HN- A_1-A_2-...-A_{i-1}-Arg, the singly ionized cationic species of $(M+H)^+$ is measured at m/z=M+1 with relatively strong intensity of $I((M+H)^+)$ in the spectrum of the cationic species measured by means of MALDI-TOF-MS, whereas the singly anionic ionized species of (M-H) is measured at m/z=M-1 with relatively weak intensity of $I((M-H)^-)$ in the spectrum of the anionic species measured by means of MALDI-TOF-MS.

In addition, in the case of the peptide fragment with molecular weight of M having N-terminal amino group ($-NH_2$) and no Arg as its C-terminal amino residue: H_2N-A_1-A_2-...-A_{i-1}-Arg (where A_i is another amino acid than Arg), or the N-terminal acetylated peptide fragment with molecular weight of M having no Arg as its C-terminal amino residue: CH_3CO-HN- A_1-A_2-...-A_{i-1}-Arg (where A_i is another amino acid than Arg), the singly anionic ionized species of $(M-H)^-$ is measured at m/z=M-1 with relatively strong intensity of $I((M-H)^-)$ in the spectrum of the anionic species measured by means of MALDI-TOF-MS, whereas the singly ionized cationic species of $(M+H)^+$ is measured at m/z=M+1 with relatively weak intensity of $I((M+H)^+)$ in the spectrum of the cationic species measured by means of MALDI-TOF- MS.

Therefore, in a case when the peak of the singly ionized cationic species of $m/z=M_{obs}+1$ is measured in the spectrum of the cationic species with relatively strong intensity, whereas the peak of the anionic species of $m/z=M_{obs}+1$ is measured in the spectrum of the anionic species with relatively weak intensity, the peak of the singly ionized cationic species of $m/z=M_{obs}+1$

with relatively strong intensity is reasonably assigned as the peak of singly ionized cationic species of $(M_{obs}+H)^+$ generated from the peptide fragment of M_{obs} having Arg as its C-terminal amino residue: H_2N-A_1-A_2-...-A_{i-1}-Arg or CH_3CO-HN- A_1-A_2-...-A_{i-1}-Arg, and the peak of the anionic species of m/z= M_{obs} -1 with relatively weak intensity is also reasonably assigned as the peak of singly ionized anionic species of $(M_{obs}-H)^-$ generated from the peptide fragment of M_{obs} having Arg as its C-terminal amino residue: H_2N-A_1-A_2-...-A_{i-1}-Arg or CH_3CO-HN- A_1-A_2-...-A_{i-1}-Arg.

In a case when the peak of the anionic species of m/z=M_{obs} -1 is measured in the spectrum of the anionic species with relatively strong intensity, whereas the peak of the singly ionized cationic species of m/z= M_{obs} +1 is measured in the spectrum of the cationic species with relatively weak intensity, the peak of the singly ionized cationic species of m/z= M_{obs} +1 with relatively weak intensity is reasonably assigned as the peak of singly ionized cationic species of $(M_{obs}+H)^+$, generated from the peptide fragment of M_{obs}, having no Arg as its C-terminal amino residue: H_2N-A_1-A_2-...-A_{i-1}-Arg or CH_3CO-HN- A_1-A_2-...-A_{i-1}-Arg (where A_i is another amino acid than Arg), and the peak of the anionic species of m/z=M_{obs} -1 I with relatively strong intensity is reasonably assigned as the peak of singly ionized anionic species of $(M_{obs}-H)^-$ generated from the peptide fragment of M_{obs} having no Arg as its C-terminal amino residue: H_2N-A_1-A_2-...-A_{i-1}-Arg or CH_3CO-HN- A_1-A_2-...-A_{i-1}-Arg (where A_i is another amino acid than Arg).

The group of the peaks of singly ionized cationic species having considerable intensity selected in the first step is reasonably divided into a subgroup of the peak of the singly ionized cationic species generated from the peptide fragment having Arg as its C-terminal amino residue. H_2N-A_1-A_2-...-A_{i-1}-Arg or CH_3CO-HN- A_1-A_2-...-A_{i-1}-Arg and another subgroup of the peak of

the singly ionized cationic species generated from the peptide fragment having no Arg as its C-terminal amino residue: H_2N-A_1-A_2-...-A_{i-1}-Arg or CH_3CO-HN- A_1-A_2-...-A_{i-1}-Arg (where A_i is another amino acid than Arg). The group of the peaks of singly ionized anionic species having considerable intensity selected in the first step is also reasonably divided into a subgroup of the peak of the singly ionized anionic species generated from the peptide fragment having Arg as its C-terminal amino residue: H_2N-A_1-A_2-...-A_{i-1}-Arg or CH_3CO-HN- A_1-A_2-...-A_{i-1}-Arg and another subgroup of the peak of the singly ionized anionic cationic species generated from the peptide fragment having no Arg as its C-terminal amino residue: H_2N-A_1-A_2-...-A_{i-1}-Arg or CH_3CO-HN- A_1-A_2-...-A_{i-1}-Arg (where A_i is another amino acid than Arg).

In using the methodology of the combination of the prior art, a case where only the spectrum of the cationic species measured by means of MALDI-TOF-MS is used for analysis, it is hard to reasonably distinguish the peak of singly ionized cationic species generated from the peptide fragment having Arg as its C-terminal amino residue: H_2N-A_1-A_2-...-A_{i-1}-Arg or CH_3CO-HN- A_1-A_2-...-A_{i-1}-Arg from the peak of singly ionized cationic species generated from the peptide fragment having no Arg as its C-terminal amino residue: H_2N-A_1-A_2-...-A_{i-1}-Arg or CH_3CO-HN- A_1-A_2-...-A_{i-1}-Arg (where A_i is another amino acid than Arg). In this case if only the spectrum of the anionic species measured by means of MALDI-TOF-MS is used for analysis, it is very hard to reasonablv distinguish the peak of singly ionized anionic species generated from the peptide fragment having no Arg as its C-terminal amino residue: H_2N-A_1-A_2-...-A_{i-1}-Arg or CH_3CO-HN- A_1-A_2-...-A_{i-1}-Arg (where A_i is another amino acid than Arg) from the peak of singly ionized anionic species generated from the peptide fragment having Arg as its C-terminal amino residue: H_2N-A_1-A_2-...-A_{i-1}-Arg or CH_3CO-HN- A_1-A_2-...-A_{i-1}-Arg. The same

problem also exists if one has a peak of a singly ionized cationic species and a singly anionic species.

However, by following the steps of the present invention, in which step 1 to step 9 are followed, the skilled artisan can reasonably identify the couple of the singly ionized cationic species and singly ionized anionic species generated from the same peptide fragment and also to reasonably classify the identified couple of the singly ionized cationic species and singly ionized anionic species into the subgroup of a couple of the singly ionized cationic species and singly ionized anionic species generated from peptide fragment having Arg as its C-terminal amino residue and the subgroup of a couple of the singly ionized cationic species and singly ionized anionic species generated from peptide fragments having no Arg as its C-terminal amino residue using both the spectrum of cationic species and the spectrum of anionic species.

It is to be noted that the examples of analysis based on the one of the spectrum of cationic species or one of the spectrum of anionic species suggested by the combination of Tsugita, Covey and Xu are results of analysis of known proteins or peptidoglycans whose amino acid sequence is known. Thus, identification and assignment of cationic species or anionic species of the peptide fragments measured is made easily based on the known amino acid sequence of the known proteins or peptidoglycans. However, in the situation where the amino acid sequence of the target protein is not known, the combination of Tsugita, Covey and Xu fails to teach any method to reasonably identify and assign the cationic species or anionic species generated from the peptide fragments of the target protein only based on the measured m/z and intensity of the cationic species or anionic species.

The assignment of the $(2M+2H)^{2+}$ species and $(2M-2H)^{2-}$ species to the same peak leaves methods, as suggested by Tsugita, Covey and Xu, where only the spectrum of one species is

measured. Using this measurement process one of ordinary skill in the art is unable to distinguish the peaks of singly ionized anionic species from groups of peaks of doubly ionized anionic species. The inability of the combination of the Tsugita, Covey and Xu references to distinguish the peaks of singly ionized anionic species from groups of peaks of doubly ionized anionic species makes the determination of the amino acid sequence more difficult, and less accurate. In the case where the amino acid sequence of the protein is not known, the combination of the Tsugita, Covey and Xu references do not teach a method of how to reasonably identify and assign an unknown sequence of amino acids based on the measured m/z and intensity of the cationic species or anionic species. On the other hand, the method of the claimed invention quickly and accurately identifies and assigns the unknown sequence of amino acids solely based on the measured m/z and intensity of the cationic species and anionic species. Therefore, the claimed invention has an advantage not taught or suggested by the present invention.

Assuming, *pro arguendo*, that the combination of references do teach a method identifying and assigning the unknown sequence of amino acids solely based on the measured m/z and intensity of the cationic species and anionic species, the claimed invention does this process more quickly and more accurately because more information is collected about each protein sample. The method of the claimed invention measures both singly and doubly charged cationic and anionic species of each amino acid in one step, without the need for further processing. The larger amount of information collected during the method of the claimed invention results in a faster and more efficient sequence determination, because no further steps are required for clarification or further measurement, and a more accurate sequence determination results from the greater amount of data measured for each amino acid and the

accurate measurement of both the singly and doubly charged anionic and cationic species at one time.

None of the references, Tsugita, Covey or Xu teach or suggest measuring the mass/charge of the cationic species of $(M+H)^+$ as well as the mass/charge measurement for the anionic species of $(M-H)^-$, as recited in Claims 1, 18 and all claims depending therefrom of the present application. Therefore, the combination of Tsugita, Covey and Xu does not teach or suggest this recited element. Since the combination of Tsugita, Covey and Xu does not teach or suggest measuring the mass/charge of the cationic species of $(M+H)^+$ as well as the mass/charge measurement for the anionic species of $(M-H)^-$, the combination of references do not render the claimed invention obvious. Thus, it is respectfully requested that the rejection of Claims 1, 2 and 4-6 under 35 U.S.C. §103(a) be withdrawn.

Claim 3 stands rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Tsugita in view of Covey and Xu, in further view of Harris. The deficiencies of Tsugita, Covey and Xu are discussed above. Harris does not cure these deficiencies. This rejection should be withdrawn based on the comments and remarks herein.

Harris teaches a method of using the $(M+H)^+$ ions from the trypsin autolysis fragments as mass calibrants in the positive-ion mode MALDI-TOF based analysis. Harris fails to provide any experimental evidence suggesting that the $(M-H)^-$ ions from the trypsin autolysis fragments will be successfully used as mass calibrants in the negative-ion mode MALDI-TOF based analysis. Further, Harris does not teach measuring the molecular weight of the cationic species of $(M+H)^+$ as well as the molecular weight measurement for the anionic species of $(M-H)^-$, as recited in Claim 1, from which Claim 3 depends.

Further, the combination of Tsugita, Covey, Xu and Harris fail to teach at least the following elements of Claim 1, from which Claim 3 depends. The combination of references fails to teach measuring the mass/charge of the cationic species of $(M+H)^+$ as well as the mass/charge measurement for the anionic species of $(M-H)^-$ and the use thereof for determining the sequences of the amino acid, as claimed, in an unknown peptide.

None of the references, Tsugita, Covey, Xu or Harris, alone or in combination, teach measuring the mass/charge of the cationic species of $(M+H)^+$ as well as the mass/charge measurement for the anionic species of $(M-H)^-$, as recited in Claims 1, 18 and all claims depending therefrom of the present application. Therefore, the combination of Tsugita, Covey, Xu and Harris does not teach or suggest this recited element. Since the combination of Tsugita, Covey, Xu and Harris does not teach or suggest measuring the mass/charge of the cationic species of $(M+H)^+$ as well as the mass/charge measurement for the anionic species of $(M-H)^-$, the combination of references do not render the claimed invention obvious. Thus, it is respectfully requested that the rejection of Claim 3 under 35 U.S.C. §103(a) be withdrawn.

Claims 7-17 stand rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Tsugita in view of Covey and Xu and in further view of Vogt. The deficiencies of Tsugita, Covey and Xu are discussed above and herein below. Vogt does not cure these deficiencies. This rejection should be withdrawn based on the comments and remarks herein.

Vogt teaches a process for the preparation of a high reactive gel-suspension of carboxymethyl cellulose (CMC), in which the polymer (carboxymethyl cellulose) is treated in a dipolar-aprotic solvent, such as N,N-dimethylacetamide and dimethylsulfoxide (DMSO), with p-toluene-sulfonic acid. Vogt is purely a purification process and it has nothing at all to do with determining amino acid sequence. Vogt does not teach measuring the mass/charge of the

cationic species of $(M+H)^+$ as well as the mass/charge measurement for the anionic species of $(M-H)^-$ as recited in Claim 1, 18 and all claims depending therefrom of the present application.

Further, the combination of Tsugita, Covey, Xu and Vogt fail to teach or suggest at least the following elements of Claim 1, from which Claim 7-17 depend. The combination of references fails to teach measuring the mass/charge of the cationic species of $(M+H)^+$ as well as the mass/charge measurement for the anionic species of $(M-H)^-$.

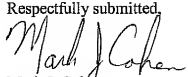
Therefore, the combination of Tsugita, Covey, Xu and Vogt does not teach or suggest this recited element. Since the combination of Tsugita, Covey, Xu and Vogt does not teach measuring the mass/charge of the cationic species of $(M+H)^+$ as well as the mass/charge measurement for the anionic species of $(M-H)^-$, the combination of references do not render the claimed invention obvious. Thus, it is respectfully requested that the rejection of Claims 7-17 under 35 U.S.C. §103(a) be withdrawn.

Further, there is no rejection of Claim 18 in the Detailed Action of the present Official Action. Issuance of Claim 18 is earnestly solicited.

For at least the reasons set forth in the foregoing discussion, Applicants believe that the Application is now allowable, and respectfully requests that the Examiner reconsider the rejection and allow the Application. Should the Examiner have any questions regarding this

Amendment, or regarding the Application generally, the Examiner is invited to telephone the undersigned attorney.

Respectfully submitted,



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